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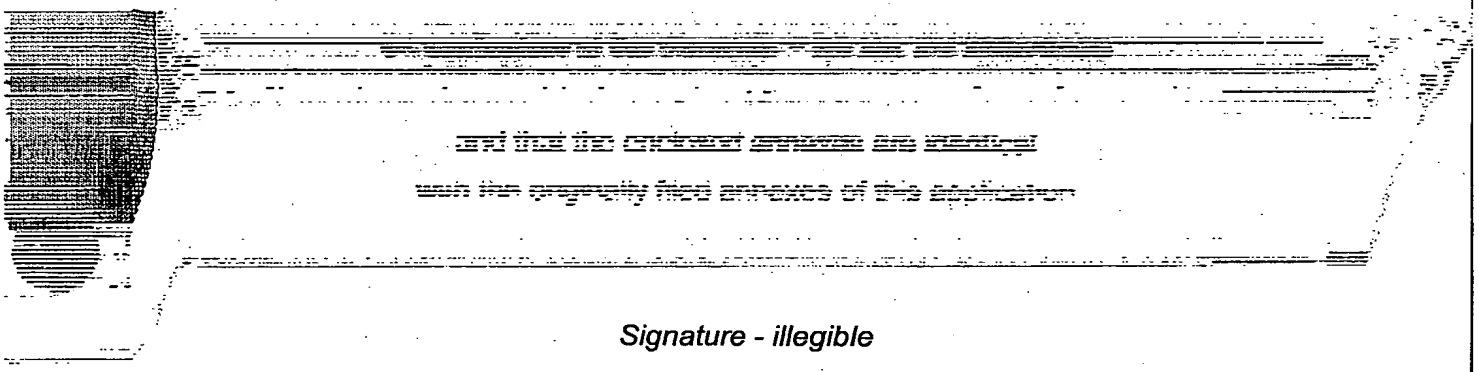

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Also the authors of patent EP 680320 pointed out that the substance atorvastatin has insufficient stability. It is stated in the specification of the said patent that it is an unstable substance sensitive to heat, humidity, low pH of the environment, and light, particularly UV radiation. A composition whose main feature are basic inorganic substances is the solution to this problem. Hydroxides, oxides or carbonates are the preferred anions. As to cations, most often calcium, magnesium, and lithium salts are stated. Calcium carbonate is stated as the best solution. Antioxidants of anisole or ascorbate type are also added to the recommended composition.

In WO 00/34525, the stabilization of a dosage form is solved by adding buffers, especially citrates.

WO 01/76566 solves the stabilization of a dosage form by adding a basic polymer containing amino or amido groups, for example polyvinylpyrrolidone.

WO 01/93859 solves the stabilization of HMG-CoA inhibitors, and among them also of atorvastatin, using a substance capable of binding and neutralizing carbon dioxide. Carbon dioxide is, according to the authors of the application, the most important factor leading to the instability of the product. Its effect is ascribed to the lowering of pH, which results in the decomposition of hydroxyacids particularly to their lactones. It is pointed out that gastric troubles may be caused if a medicine with a high content of alkaline substances is administered to patients. This fact limits the possibility of improving the stability by adding a stabilizer to the dosage form.

WO 02/072073 shows the relation between the pK_a value of atorvastatin and the pH value of an aqueous solution of a solid dosage form. According to the quoted application, the dosage form should contain such ingredients which would cause the pH of solution to reach a value not lower than $pK_a + 1$.

Accordingly, it follows from the prior art that the main methods how to solve the problem of the stability of atorvastatin in a dosage form were either increase of the pH of the dosage form, or prevention of the lowering of the pH by CO_2 contained in the atmosphere.

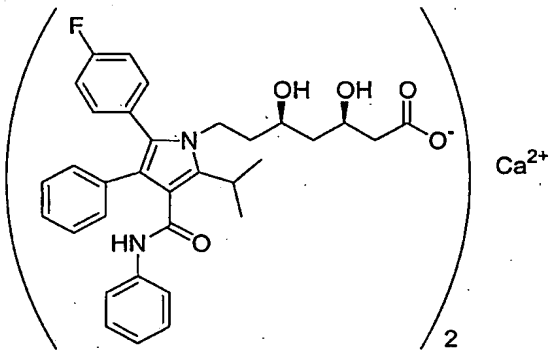
Stabilization of Atorvastatin by Trapping Free Oxygen

Technical Field

This invention relates to stabilization of the extremely unstable substance atorvastatin, in its crystalline, but particularly amorphous state. The stabilization can be used for the pure substance, but also for the substance in solid or liquid dosage forms.

Background Art

The hemicalcium salt of (3*R*,5*R*) 7-[3-phenyl-4-phenylcarbamoyl-2-(4-fluorophenyl)-5-isopropylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid of formula I



(I)

known under the non-proprietary name atorvastatin (I), in the text sometimes called the calcium salt of atorvastatin, is produced according to published patents (US patents 4,681,893 and 5,273,995). The said drug is an important representative of hypolipidemic and hypocholesteric drugs.

Atorvastatin can exist in various crystalline forms or in an amorphous form. The preparation of various polymorphs is described in published patents (US 5,969,156; US 6,121,461; WO 03/004470 and WO 01/36384), the amorphous form in patent US 6,087,511. The crystalline forms are, according to the above-mentioned patents, much more stable than the amorphous form.

However, new facts have now surprisingly shown that degradation of atorvastatin, which does not contain this system of conjugated bonds, is also caused by atmospheric oxygen. Moreover, it has been shown that the usual solution to the problem – pharmaceutical composition containing a substance susceptible to oxidation –, that is the use of a formulation with an antioxidant, has, in the case of atorvastatin (stated, for example, in EP 680320) failed (example 6 of this document).

Disclosure of Invention

The purpose of this invention resides in the protection of atorvastatin from oxidation by atmospheric oxygen. The protection applies to the chemical substance in all crystalline forms, including the amorphous form, as well as to dosage forms when using atorvastatin in any polymorphous form, or in a solution.

The mentioned protection from oxidation subsists in the fact that free oxygen is trapped and eliminated in the container of atorvastatin, whether in the form of the active substance or in the form of a pharmaceutical preparation produced from that substance. The elimination of free oxygen is carried out in the space which is separated from atorvastatin best by a partially permeable barrier.

As to the dosage form, a special formulation of auxiliary substances makes it possible to strengthen the protective influence of oxygen absorbers.

Detailed Description of the Invention:

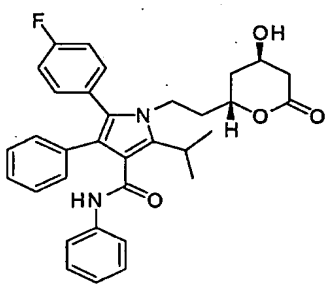
The instability of atorvastatin is, according to the above-mentioned patents, usually ascribed to the increased sensitivity to the acidity of the environment, which causes that atorvastatin may dehydrate to the lactone of atorvastatin of formula II

Despite these measures, the dosage forms of atorvastatin, and particularly if amorphous atorvastatin is in these forms, showed significant instability. Although the formation of undesirable products such as the lactone of atorvastatin was prevented, the formation of other unknown substances occurred. The active substance itself, not in the dosage form, showed even worse stability. Therefore, it was necessary to store and transport amorphous atorvastatin at about –20 °C. Naturally, these measures increased the costs of the said operations.

In addition to the above-mentioned methods, a new method for the preservation of substances susceptible to oxidation with the use of substances trapping air oxygen, often called oxygen absorbers, has been developed. Mitsubishi Gas Chemical (Tokyo, Japan) have developed bags absorbing oxygen based on a reaction of iron under the trade name Ageless (Yoshikawa, Y., Amemiya, A.; Komatsu, T.; Inoue, Y.; Yuyama, M., Oxygen Absorbent for Food Packaging. Jpn. Kokai Tokkyo Koho, Showa 56-33980, 1978). Similar products are also offered, for example, by Multisorb Technologies, Inc. under the trade name Fresh Pax™ or by Standa Industry under the trade name ATCO.

Many products are available nowadays. They are based on humidity-activated oxygen absorbers, self-activating absorbers, ultraviolet-radiation-activated absorbers, radiation-activated absorbers, microwaves-activated absorbers, absorbers activated by a combination of activation processes, or absorbers not requiring any activation.

In patent application US 2002/0132359, the use of these absorbers for pharmaceutical preparations sensitive to oxygen is applied for protection. The application is carried out in a blister packing where the absorber is situated between the lid and the blister itself. The application further informs that it is very difficult to find out which of the substances will be susceptible to oxidation. The problem subsists in the fact that often the oxidation does not follow the classical Arrhenius equation, and that is why accelerated stability tests, which are successfully used for other decomposition reactions, fail. The patent application further contains a list of some pharmaceutical substances which could be sensitive to oxygen. HMG–CoA inhibitors simvastatin or lovastatin are the most relevant ones among them. Both these substances contain a system of conjugated double bonds in a carbocyclic system, which can result in sensitivity to oxygen.



(II)

Lactonization is an acid-catalyzed process proceeding probably via a free dihydroxyacid of atorvastatin. Therefore, the solution subsists in adding basic substances to the dosage forms.

However, we have showed, with the help of exactly controlled experiments, that the instability is also caused by oxidation by atmospheric oxygen. Particularly amorphous atorvastatin shows significant instability towards oxidation, whereas crystalline forms are somewhat more stable. However, this is caused by statistical factors because a substance firmly incorporated in a crystal lattice has a lesser probability to react with atmospheric oxygen than a substance in an amorphous form (Stephen R. Byrn: *Solid State Chemistry of Drugs*, Academic Press, 1982). Therefore, the oxidative decomposition of crystalline forms of atorvastatin is slower than that of the amorphous form. This is also important for dosage forms because, for example, mechanical stress when producing tablets may lead to partial collapse of the crystalline structure causing instability of the dosage form.

The following experiments were carried out in order to determine the instability of atorvastatin. The purpose was to find out which factors lead to the degradation of the product.

1. Set of stress tests. The stock solution of atorvastatin (2 ml) was gradually subjected to the following experiments:

a. boil (24 hr) with 2 ml of 0.2 N hydrochloric acid,
b. boil (24 hr) with 2 ml of 0.2 N acetic acid,
c. boil (24 hr) with 2 ml of 0.2 N sodium hydroxide,
d. boil (24 hr) with 2 ml of 4% hydrogen peroxide,
e. boil (24 hr) with 2 ml of water,
f. irradiation with UV radiation (5 hr),
g. irradiation with visible light (24 hr),

h. solid substance heated for 24 hr at 100 °C,

i. solid substance subjected to UV radiation (5 hr),

j. solid substance subjected to visible light (24 hr).

The results of analytical assessment (HPLC) are summarized in the following table (Tab. 1):

Table 1

Conditions	Content of Atorvastatin [%]
24 hr boil in 0.1 N HCl	2.1
24 hr boil in 0.1 N AcOH	83.2
24 hr boil in 0.1 N NaOH	72.0
24 hr boil in 2% H_2O_2	54.8
24 hr boil in water	88.4
5 hr under UV in water	64.3
24 hr in the light	104.5
substance 24 hr, 100 °C	96.3
substance 5 hr, UV	99.3
substance 24 hr, light	96.5

What could particularly be seen from the results was instability in the acid environment. Furthermore, the substance decomposed significantly also in a solution when subjected to UV radiation, which is in conformity with literary data (*Tetrahedron* 49,10,1979-1984,1993).

Decomposition by hydrogen peroxide turned out to be another significant factor.

In order to make the carried-out experiments more precise, stability test of solid amorphous atorvastatin and of several selected dosage forms were established. As to containers, polyethylene (PE) and an aluminium foil with a sealable PE layer (Al + PE) were used. Some of the stability data were determined in nitrogen atmosphere (N_2).

The results of these stability tests are summarized in Table 2.

Table 2

Sample	Total Impurities [%]
entry	0.21
3 mths, container 2x PE (5 °C)	0.39
3 mths, container PE+Al (5 °C)	0.34
3 mths, container PE+Al+N_2 (5 °C)	0.34
3 mths, container 2x PE (25 °C)	0.77
3 mths, container PE+Al (25 °C)	0.63
3 mths, container PE+Al+N_2 (25 °C)	0.43
6 mths, container 2x PE (5 °C)	0.83
6 mths, container PE+Al (5 °C)	0.71
6 mths, container PE+Al+N_2 (5 °C)	0.44

It follows from the analyses that the use of aluminium foil and nitrogen atmosphere significantly increases the stability of the product. That would indicate that particularly atmospheric oxygen is responsible for the low stability of the product. The results were similar also for dosage forms.

In order to precisely determine the decomposition mechanism, the following experiments examining only the oxidative decomposition of atorvastatin were carried out. A recent publication /*Pharmaceutical Development and Technology*, 7(1), 1-32 (2002)/ described a set of experiments which are suitable for the recognition of oxidation of substances and for the determination of its mechanism.

The following experiments were carried out:

a. oxidation of a 1% solution of atorvastatin in the system of ethyl acetate – acetonitrile (1:1) at 40 °C using a radical initiator (2,2'-azobiscyanopentanoic acid) at a pressure of 10 atmospheres of oxygen;

b. oxidation of a 1% solution of atorvastatin in the system of ethyl acetate – acetonitrile (1:1) at 40 °C without a radical initiator at a pressure of 10 atmospheres of oxygen;

c. control experiment in the system of ethyl acetate – acetonitrile (1:1) at 40 °C using a radical initiator (2,2'-azobiscyanopentanoic acid) in an inert atmosphere of argon.

The results are summarized in Table 3.

Table 3

Sample	Total Impurities [%]
without load	0.49
oxidation (with initiator) 24 hr	6.42
oxidation (with initiator) 48 hr	24.37
oxidation (with initiator) 72 hr	30.93
24 hr (argon)	0.98
48 hr (argon)	1.38
oxidation - 24 hr without initiator	9.27

It follows from the results that atmospheric oxygen itself can oxidize atorvastatin and no radical initiator is necessary for the oxidation. The control experiment in an inert atmosphere of argon showed a small increase in the amount of the lactone of atorvastatin caused by increased temperature and slightly acidic radical initiator.

The comparison of the profile of impurities from stability tests (Table 2) with the profile of impurities created by oxidation using HPLC-MS was another result of the experiment. It was shown that all significant impurities the amount of which increased during the stability tests, with the exception of the lactone of atorvastatin, are formed by oxidation. On the basis of this knowledge of oxidative decomposition, a solution was looked for which would prevent the contact of the substance itself or the substance in a dosage form with atmospheric oxygen. According to our, and also in literature published /K.C. Waterman, M.C. Roy: Pharmaceutical Development and Technology, 7 (2), 227-234 (2002)/, experience, it is very difficult, when packing the dosage form, to reach the residual concentration of oxygen lower than 2 – 3 % without using a vacuum step. This is a sufficient amount to cause oxidation of the product still occur. An especially suitable method how to achieve a lower concentration of oxygen (as low as below the value of 0.1 %) is the use of oxygen absorbers. Therefore, furher experiments

were carried out with the use of oxygen absorbers. The experiments were carried out with oxygen absorbers Ageless™ from Mitsubishi Gas Chemical and ATCO from Standa Industry. Many other commercially available absorbers can also be advantageously used; for example FreshPax™ (Multisorb Technologies), O-Buster™ (Hsiao Sung Non-Oxygen Chemical Co), Biotika Oxygen Absorber (Biotika) and the like.

The use of oxygen absorbers especially for the protection of foods, but also of chemical substances, from oxidation is well-known from published patents (US 4,287,995; US 5,143,763; US 5,839,593).

As to the method of their activation, oxygen absorbers can be divided into the following groups: humidity-activated absorber, self-activating absorber, ultraviolet-radiation-activated absorber, radiation-activated absorber, microwaves-activated absorber, absorber activated by a combination of activation processes, or absorber without necessity of activation.

Absorbers which do not need water for their activation are advantageous for the method of stabilization according to this invention. Particularly advantageous are those which do not need activation at all.

The above-mentioned results show that oxidative degradation is an important factor mainly for amorphous atorvastatin and this fact has to be taken into consideration when storing the substance or the final dosage form. We have shown that the use of oxygen absorbers significantly improves the storability of amorphous atorvastatin (examples 1 and 2). It clearly follows from the results that the protection of atorvastatin from atmospheric oxygen completely prevents its decomposition. When using oxygen absorbers, the substance can be then stored at 25 °C without any limitations, which means, in comparison with the storage at a lower temperature, a significant decrease of costs. The substance can also be stored in other containers which let oxygen through partially with the final concentration of oxygen lower than 1 %, ideally lower than 0.1 %. The substance can also be advantageously packed in an inert atmosphere, which makes the lifetime of the oxygen absorber longer and the initial exothermic reaction when trapping oxygen by the absorption bag milder. The needed capacity of the absorption bag and the resultant equilibrium concentration of oxygen (in ppm) can be calculated from the following equation (Vinod Daniel, Frank L. Lambert: *Waac Newsletter* 15, 2, 1993, 12-14, 1993):

$[O_2] = L / 12.7\ C$

wherein L is the leakage rate of oxygen out of the container in ppm/day, C is absorbance, which is the ratio of the capacity of the absorption bag and the total volume of the container.

This invention also solves the problem of the stabilization of atorvastatin in dosage forms, particularly in the form of tablets or capsules. The described dosage forms of atorvastatin contain approximately 1 to 60 % by weight, preferably 3 to 20 % by weight of the active substance and several auxiliary substances with various functions, especially to help to release the active substance in a patient's body at the desired rate, to stabilize the dosage form against chemical decomposition or mechanical influences. In order to stabilize atorvastatin in the dosage form, it is usually recommended to add a basic substance, calcium carbonate being mentioned as the most preferable one.

Surprisingly, it has been shown that there is a close relation between atmospheric oxygen and a suitable formulation. Some formulations, which are relatively successful when storing the dosage form with a normal access of oxygen, turn out to be unsuitable for oxygen trapping. On the contrary, those that are less suitable in normal conditions, strengthen the stabilization influence of oxygen trapping. Products which are in no immediate relation to the oxidation are at fault. The lactone of atorvastatin of the above-mentioned formula II is an example. The basic action of calcium carbonate prevents, in normal conditions, the acid-catalyzed reaction and limits the formation of the lactone (EP 680320). When trapping oxygen, this effect of calcium carbonate is limited and the concentration of the lactone increases with time. The use of a base like magnesium oxide or hydroxide is usually considered less suitable. In normal conditions, i.e., with the access of oxygen, the formulation with a magnesium base leads not only to the increase in the amount of usual impurities, but also to the formation of many impurities which are not identified when using calcium carbonate. On the contrary, when trapping oxygen, a base of this type strengthens the stabilization effect described above for the 100% substance. It is again a case of complex stabilization, i.e., not only mere limitation of apparent oxidation products. Therefore, stabilization of atorvastatin by combining the effects of oxygen absorbers and magnesium oxide is considered an especially advantageous embodiment according to this invention.

This invention is elucidated in greater detail in the following working examples. These examples are of an illustrative nature only and do not limit the scope of the invention in any way.

Examples

Example 1

Amorphous atorvastatin (1 g) was sealed in a sealable aluminium foil together with the oxygen absorber Ageless® Z100 (Mitsubishi) or with the absorber of oxygen and carbon dioxide Ageless® E100 (Mitsubishi) and the sample was heated at 80 °C for 72 hr. The reference sample was prepared and heated in the same way without the use of absorbers. The results of HPLC analyses are summarized in Table 4.

Table 4

Conditions	Total Impurities [%]	Lactone of Atorvastatin [%]
without load	0.49	0
reference sample	1.32	0.33
Ageless® Z100	0.64	0.19
Ageless® E100	0.60	0.14

Example 2

Amorphous atorvastatin (1 g) was sealed in a sealable aluminium foil together with the oxygen absorber Ageless® Z100 (Mitsubishi) or with the absorber of oxygen and carbon dioxide Ageless® E100 (Mitsubishi) and the sample was heated at 40 °C for 1.5 months. The reference sample was prepared and heated in the same way without the use of absorbers. The results of HPLC analyses are summarized in Table 5.

Table 5

Conditions	Total Impurities [%]	Lactone of Atorvastatin (%)
without load	0.49	0
reference sample	1.55	0.22
Ageless® Z100	0.36	0
Ageless® E100	0.46	0.06

Example 3

Tablets having the composition described in Table 6

Table 6

Composition of the Tablets	Amount (mg)
calcium salt of atorvastatin	20.0
lactose monohydrate	42.0
microcrystalline cellulose	60.0
calcium carbonate	88.0
hydroxypropyl cellulose	30.0
pregelatinized corn starch	30.0
polysorbate	1.0
talc	1.5
sodium salt of crosscarmelose	6.0
calcium stearate	0.5

were coated with a usual lacquer containing hydroxypropylmethyl cellulose and sealed together with the absorption bag Ageless® Z100 (Mitsubishi) in an aluminium foil and heated at 80 °C for 72 hours. The reference sample was prepared and heated in the same way without the use of oxygen absorber. The results of HPLC analyses are summarized in Table 7.

Table 7

Conditions	Total Impurities [%]	Lactone of Atorvastatin [%]
without load	0.60	0.03
reference sample	4.21	2.58
Ageless® Z100	1.98	1.13

Example 4

Tablets containing 20 mg of the amorphous form of calcium salt of atorvastatin having the composition described in Table 8

Table 8

Composition of the Tablet	Amount (mg)
calcium salt of atorvastatin	20.0
lactose monohydrate	42.0
microcrystalline cellulose	60.0
calcium carbonate	88.0
hydroxypropyl cellulose	30.0
pregelatinized corn starch	30.0
polysorbate	1.0
talc	1.5
sodium salt of crosscarmelose	6.0
calcium stearate	0.5

were coated with a usual lacquer containing hydroxypropylmethyl cellulose and filled into a glass bottle of a volume of 20 ml (reference sample). In the course of other experiments, the oxygen absorber Ageless® Z100 (Mitsubishi), or the absorber of oxygen and carbon dioxide Ageless® E100 (Mitsubishi), or the oxygen absorber Ageless® Z100 (Mitsubishi) and a desiccant were added into the bottle besides the tablets. The bottles were closed using a HDPE cap and subjected to 40 °C and 75% RH for 1.5 months. The arrangement of the experiment and the results of HPLC analyses are summarized in Table 9.

Table 9

Conditions	Total Impurities [%]	Lactone of Atorvastatin [%]
without load	0.49	0
reference sample	2.38	0.20
Ageless® Z100	1.09	0.41
Ageless® E100	1.10	0.48
Ageless® Z100 + desiccant	0.87	0.33

Example 5

Tablets containing 20 mg of the amorphous form of calcium salt of atorvastatin having the composition described in Table 10

Table 10

Composition of the Tablet	Amount (mg)
calcium salt of atorvastatin	20.0
lactose monohydrate	49.6
microcrystalline cellulose	148.0
magnesium oxide	14.0
hydroxypropyl cellulose	28.0
polysorbate	9.0
sodium salt of crosscarmelose	9.0
magnesium stearate	1.4
silicon dioxide	1.0

were coated with a usual lacquer containing hydroxypropylmethyl cellulose, filled into a glass bottle of a volume of 20 ml together with the absorption bag Ageless® Z100 (Mitsubishi) and heated at 40 °C and 75% RH for 1.5 months. The reference sample was prepared in the same

way without adding the oxygen absorber. The results of HPLC analyses are summarized in the following table (Tab. 11).

Table 11

Conditions	**Total Impurities [%]**	**Lactone of Atorvastatin [%]**
without load	0.79	0
reference sample	2.12	0
Ageless® Z100	0.84	0

Example 6

In order to find out stability of the dosage form, mixtures of the amorphous form of atorvastatin with a basic component and antioxidants were made. These mixtures were filled into glass bottles of a volume of 20 ml, closed using a HDPE cap, and heated at 40 °C and 75% RH for 6 weeks. The composition of the said mixtures and the results of HPLC analyses are summarized in Table 12.

Table 12

Mixture Number	Mixture Composition	Component Ratios	Total Impurities [%]	Lactone of Atorvastatin [%]
2	calcium salt of atorvastatin	1	2.03	0.05
	calcium carbonate	3		
5	calcium salt of atorvastatin	1	4.04	1.12
	calcium carbonate	3		
	vitamin E	1		
6	calcium salt of atorvastatin	1	4.53	1.58
	calcium carbonate	3		
	β-carotene	1		
9	calcium salt of atorvastatin	1	4.71	1.29
	calcium carbonate	3		

	sodium ascorbate	1		

It follows from the above experiment that the use of antioxidants, routinely used in the pharmaceutical industry, does not prevent oxidation processes in the dosage form.

CLAIMS

1. A method of stabilization of atorvastatin by storing in a closed container *characterized in that* free oxygen is systematically trapped and eliminated in said container, the process of trapping and elimination proceeding in a space separated from the substance to be stabilized.

2. The method according to claim 1 *characterized in that* practically 100% substance is stabilized in this way, that is without adding other auxiliary substances inhibiting decomposition reactions.

3. The method according to claim 1 *characterized in that* atorvastatin is in a mixture containing also magnesium oxide in the amount of 0.1 to 50 % by weight.

4. The method according to any of the preceding claims *characterized in that* atorvastatin is predominantly in an amorphous form.

5. The method according to claims 1, 3 or 4 *characterized in that* the method stabilizes a drug in the form of tablets or capsules containing atorvastatin in the amount of 1 to 60 % by weight.

6. The method according to any of the preceding claims *characterized in that* the trapping and elimination of oxygen proceed in a space separated by a permeable or semipermeable barrier.

7. The method according to any of the preceding claims *characterized in that* the oxygen absorber is selected from the group including a humidity-activated oxygen absorber, a self-activating absorber, an ultraviolet-radiation-activated absorber, a radiation-activated absorber, a microwaves-activated absorber, an absorber activated by a combination of activation processes, or an absorber without necessity of activation.

8. The method according to claim 7 *characterized in that* oxygen is trapped by means of self-activating absorbers.

9. The method according to claims 1 - 8 *characterized in that* the capacity of the oxygen-trapping device is selected in such a way that the resultant concentration of oxygen in

the surroundings of atorvastatin or of the drug produced therefrom is lower than 1 % by volume, preferably 0.1 % by volume.

10. The method according to claims 3 and 5 *characterized in that* the dosage form is constituted by 3 - 20 % by weight of atorvastatin, 5 - 30 % by weight of magnesium oxide, 5 - 30 % by weight of lactose, and 20 - 80 % by weight of microcrystalline cellulose.

Abstract

Title of the invention: Stabilization of Atorvastatin by Trapping Free Oxygen

A method of stabilization of atorvastatin by storing it in a closed container, wherein free oxygen is systematically trapped and eliminated, the process of trapping and elimination proceeding in a space separated from the substance to be stabilized.